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(71) Applicant (for all designated States except US):	REGENERON PHARMACEUTICALS, INC. [US/US]; 777 Old Saw Mill River Road, Tarrytown, NY 10591-6707 (US)			
(72) Inventor; and				
(75) Inventor/Applicant (for US only):	DAVIS, Samuel [US/US]; Apartment #B2, 332 W. 88th Street, New York, NY 10024 (US).			
(74) Agents:	COBERT, Robert, J.; Regeneron Pharmaceuticals, Inc., 777 Old Saw Mill River Road, Tarrytown, NY 10591 (US) et al.			

(54) Title: MOLECULAR CLONING AND CHARACTERIZATION OF MOLECULES RELATED TO RELAXIN AND THE INSULIN FAMILY OF LIGANDS

(57) Abstract

This invention concerns the molecular cloning and characterization of nucleic acids encoding molecules, referred to as relaxin-related factors, that are related to the hormone relaxin and to the insulin family of ligands. More specifically, this invention provides an isolated nucleic acid molecule encoding relaxin-related factor-1. The invention also provides an isolated nucleic acid molecule encoding relaxin-related factor-2. The present invention also provides a nucleotide sequence that encodes a relaxin-related factor, as well as cells which are genetically engineered to produce the relaxin-related factor. The invention further provides for an isolated relaxin-related factor substantially free of other proteins, as well as for an antibody which specifically binds the relaxin-related factor. The invention also provides for pharmaceutical compositions comprising a relaxin-related factor, or an antibody which specifically binds the relaxin-related factor, and a pharmaceutically acceptable carrier, as well as for methods of treatment of a human or animal body. The invention further provides for a relaxin-related factor ligandbody which comprises a relaxin-related factor fused to an immunoglobulin constant region. The invention further provides for a method of treatment comprising administering a relaxin-related factor ligandbody to a human or animal body.

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**MOLECULAR CLONING AND CHARACTERIZATION OF MOLECULES
RELATED TO RELAXIN AND THE INSULIN FAMILY OF LIGANDS**

This application claims the priority of U.S. provisional application 60/012,016
5 filed February 21, 1996 and the priority of U.S. provisional application 60/006,221
filed November 3, 1995, the contents of each of which in its entirety is
incorporated by reference into this application. Throughout this application
various publications are referenced. The disclosures of these publications in their
entireties are hereby incorporated by reference into this application.

10 This invention concerns the molecular cloning and characterization of nucleic
acid sequences that encode molecules, hereinafter referred to as relaxin-related
factors, that are related to the hormone relaxin and to the insulin family of
ligands. More specifically, this invention provides isolated nucleic acid
15 molecules encoding relaxin-related factors. The nucleic acid molecules may be
expressed in suitable host cells and are thus useful for the production of the
relaxin-related factors and relaxin-related factor ligandbodies, as well as for the
production of probes that may be used to further screen for additional related
20 family members. The invention further provides isolated relaxin-related factors
substantially free of other proteins. The isolated relaxin-related factor proteins, as
well as derivatives thereof, may be used for the preparation of antibodies and
therapeutic compositions and as growth factors for maintaining cultures of cells
which have receptors for relaxin-related factor.

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BACKGROUND OF THE INVENTION

Relaxin is a polypeptide hormone that has been found in the female of all species
studied. Relaxin is synthesized and stored in the corpora lutea of ovaries during
pregnancy and is released into the blood stream prior to parturition. The corpus
30 luteum of pregnancy is the main source of relaxin in many species but in others
the decidua is apparently of greater importance. Hisaw suggested an important
role for relaxin in mammals through its effects in dilating the pubic symphysis,
thus facilitating the birth process. (Hisaw, F. L., Proc. Soc. Exp. Biol. Med. 23, 661-

663 (1926)). Relaxin has since been found to have many other possible roles, including preparation of the endometrium for implantation, inhibition of uterine activity in early pregnancy, remodeling of the uterine stroma during pregnancy, cervical ripening and the initiation of parturition. Relaxin's main 5 cellular action in pregnancy may be to drive collagen biosynthesis in its target organs, thus facilitating the remodeling of the connective tissue. Published PCT application WO8907945 published September 8, 1989 filed by Genentech, Inc., discloses relaxin compositions which are stated to be useful for modulating the reproductive physiology of mammals during pregnancy and parturition. Relaxin 10 has been reported to be capable of altering the nature of connective tissue, influencing smooth muscle contraction, and to have the general properties of a growth factor. It has also been reported that relaxin may influence intraocular pressure when administered in pharmacologic doses. (Kass, M.A. et al., Survey of Ophthalmology 22(3): 153-176 (1977)).

15 Although the corpora lutea of the ovary as well as decidua and placental tissues are the most likely sites for expression of relaxin-related genes, relaxin has also been found in other tissues such as prostatic fluid and testis. There is evidence in 20 pigs for relaxin gene expression and translation into protein in the theca interna cells of the preovulatory follicle, the corpus luteum of the cycle and the uterus. Relaxin has been identified in boar seminal plasma and can maintain or increase sperm motility. (See Bagnell, C.A., et al., J. Reprod. & Fert. (Supp.) 48:127-138 25 (1993) for review). It has been reported that specific, high activity human relaxin binding sites are present in discrete regions of the rat brain such that the distribution of some of these sites may be consistent with a role for relaxin in control of vascular volume and blood pressure. (Osheroff, P.L. and Phillips, H.S., Proc. Nat. Acad. Sci. (USA) 88 (15): 6413-6417 (1991)).

30 Biologically active relaxin consists of two peptide chains (known as the A and B chains) held together by one intra-chain and two inter-chain disulfide bonds. The structure thus closely resembles insulin, which has led to speculation of a common ancestral gene for these hormones (Schwabe, C., et al., Biochem.

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Biophys. Res. Commun. 75, 503-510 (1977); James, R., et al., Nature, 267:544-546 (1977)). Relaxin is considered a member of the insulin family, which also includes insulin, IGF1, IGF2 and Leydig insulin-like protein. cDNA clones for both rat and porcine relaxins have been obtained via recombinant DNA techniques. (Hudson, P., Haley, J., Cronk, M., Shine, J. and Niall, H. Nature, 291: 127-131 (1981)). Further, the molecular cloning and characterization of a gene sequence coding for human relaxin has been described. (U.S. Patent No. 4,871,460 issued October 3, 1989 to Hudson, et al.)

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SUMMARY OF THE INVENTION

This invention concerns the molecular cloning and characterization of nucleic acids encoding molecules, referred to as relaxin-related factors, that are related to the hormone relaxin and to the insulin family of ligands.

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More specifically, this invention provides an isolated nucleic acid molecule encoding relaxin-related factor-1 (RRF-1). The present invention also provides a nucleotide sequence that encodes RRF-1, as well as cells which are genetically engineered to produce RRF-1.

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This invention also provides an isolated nucleic acid molecule encoding relaxin-related factor-2 (RRF-2). The present invention also provides a nucleotide sequence that encodes RRF-2, as well as cells which are genetically engineered to produce RRF-2.

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The invention further provides for isolated RRF-1 or RRF-2 substantially free of other proteins, as well as for an antibody which specifically binds RRF-1 and an antibody that specifically binds RRF-2. The antibodies may be polyclonal or monoclonal.

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The invention also provides for a pharmaceutical composition comprising RRF-1 or RRF-2 and a pharmaceutically acceptable carrier. The invention further

provides for a pharmaceutical composition comprising an antibody which specifically binds RRF-1 or an antibody that specifically binds RRF-2 and a pharmaceutically acceptable carrier.

5 The invention further provides for a method of treatment comprising administering RRF-1 or RRF-2 to an animal or human body as well as a method of treatment comprising administering to an animal or human body an antibody which specifically binds RRF-1 or RRF-2.

10 The invention further provides for a relaxin-related factor ligandbody which comprises RRF-1 or RRF-2 fused to an immunoglobulin constant region. The invention further provides for a method of treatment comprising administering a relaxin-related factor ligandbody to a human or animal body.

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BRIEF DESCRIPTION OF THE FIGURES

FIGURE 1 - Nucleotide and deduced amino acid (single letter code) sequences of relaxin-related factor-1 (RRF-1).

20 FIGURE 2 - Comparative alignment of amino acid sequences of relaxin-related factor-1 (RRF-1) and relaxin-related factor-2 (RRF-2) with amino acid sequences of other members of the insulin family including: relaxin H1; relaxin H2; Leydig insulin-like protein (leydig); insulin; insulin-like growth factor 1 (IGF1); and insulin-like growth factor 2 (IGF2). Conserved cysteine residues are indicated by asterisks above the RRF-1 sequence. Dots indicate identical amino acid residues. 25 Dashes represent artificial insertions for purposes of alignment. Deletions ranging from 14 - 123 amino acids as well as deletions of the signal sequences have been made for purposes of alignment and are indicated in parentheses.

30 FIGURE 3 - Northern blot showing expression of message encoding relaxin-related factor-1 (RRF-1) in human testis, but not in any other human tissue tested. Lanes, from left, are Brain, Cerebellum, Thymus, Heart, Liver, Small

Intestine, Skeletal Muscle, Bone Marrow, Prostate, Testis, Ovary, Uterus, Placenta.

FIGURE 4 - Nucleotide and deduced amino acid (single letter code) sequences of relaxin-related factor-2 (RRF-2).

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FIGURE 5 - Northern blot showing expression of message encoding relaxin-related factor-2 (RRF-2) in human placenta, but not in any other human sample tested. Lanes, from left, are Cerebellum, Retina, Thymus, Heart, Lung, Spleen, Liver, Kidney, Adrenal, Pancreas, Small Intestine, Testis, Skeletal Muscle, HeLa cells, Placenta, Fetal Brain and Fetal Liver.

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DETAILED DESCRIPTION OF THE INVENTION

This invention concerns the molecular cloning and characterization of nucleic acids encoding molecules, hereinafter referred to as relaxin-related factors, that are related to the hormone relaxin and to the insulin family of ligands. More specifically, this invention provides an isolated nucleic acid molecule encoding relaxin-related factor-1 (RRF-1). The present invention also provides a nucleotide sequence that encodes RRF-1 as well as cells which are genetically engineered to produce the molecule, by e.g. transfection, transduction, infection, electroporation, or microinjection of a nucleic acid encoding RRF-1 as described herein in a suitable expression vector.

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This invention also provides an isolated nucleic acid molecule encoding relaxin-related factor-2 (RRF-2). The present invention also provides a nucleotide sequence that encodes RRF-2 as well as cells which are genetically engineered to produce the molecule, by e.g. transfection, transduction, infection, electroporation, or microinjection of a nucleic acid encoding RRF-2 as described herein in a suitable expression vector.

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The invention further provides for an isolated nucleic acid molecule encoding relaxin-related factor-1, having a sequence selected from the group consisting of:

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- (a) the nucleotide sequence comprising the coding region of relaxin-related factor-1 as set forth in Figure 1;
- (b) a nucleotide sequence that hybridizes under moderately stringent conditions to the nucleotide sequence of (a) and which encodes a molecule having the biological activity of relaxin-related factor-1; and
- (c) a nucleotide sequence which, but for the degeneracy of the genetic code would hybridize to a nucleotide sequence of (a) or (b), and which encodes a molecule having the biological activity of relaxin-related factor-1.

10 The invention further provides for an isolated nucleic acid molecule encoding relaxin-related factor-2, having a sequence selected from the group consisting of:

- (a) the nucleotide sequence comprising the coding region of relaxin-related factor-2 as set forth in Figure 4;
- (b) a nucleotide sequence that hybridizes under moderately stringent conditions to the nucleotide sequence of (a) and which encodes a molecule having the biological activity of relaxin-related factor-2; and
- (c) a nucleotide sequence which, but for the degeneracy of the genetic code would hybridize to a nucleotide sequence of (a) or (b), and which encodes a molecule having the biological activity of relaxin-related factor-2.

20 The invention also provides for a vector which comprises an isolated nucleic acid molecule encoding relaxin-related factor-1. The invention further provides for a vector which comprises an isolated nucleic acid molecule encoding relaxin-related factor-2. In one embodiment, the vector is operatively linked to an expression control sequence capable of directing its expression in a host cell. In another embodiment, the vector is a plasmid.

25 The invention further provides for a host-vector system for the production of a relaxin-related factor, polypeptide, or peptide fragment thereof which comprises a vector of the invention in a host cell. The host cell preferably may be a bacterial cell such as E. coli, or a yeast, insect or mammalian cell. The invention further provides for a method of producing a relaxin-related factor which comprises

growing cells of a host-vector system under conditions permitting production of the relaxin-related factor, and recovering the relaxin-related factor so produced.

In one embodiment of the invention the relaxin-related factor is RRF-1. In another embodiment, the relaxin-related factor is RRF-2.

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The invention further provides for an isolated relaxin-related factor substantially free of other proteins. In one embodiment, the isolated relaxin-related factor is RRF-1 and is encoded by the nucleotide sequence comprising the coding region of RRF-1 as set forth in Figure 1. In another embodiment, the isolated relaxin-related factor is RRF-2 and is encoded by the nucleotide sequence comprising the coding region of RRF-2 as set forth in Figure 4.

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The invention further provides for an antibody which specifically binds a relaxin-related factor. More specifically, the invention provides for an antibody which 15 specifically binds RRF-1 and an antibody that specifically binds RRF-2. The antibody may be a polyclonal or a monoclonal antibody.

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The invention further provides for a pharmaceutical composition comprising a relaxin-related factor and a pharmaceutically acceptable carrier. By way of non-limiting example, the relaxin-related factor may be RRF-1 or RRF-2. The invention further provides for a pharmaceutical composition comprising an antibody which specifically binds a relaxin-related factor and a pharmaceutically acceptable carrier. The relaxin-related factor that the antibody specifically binds may be RRF-1 or RRF-2.

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The invention further provides for a method of treatment comprising administering a relaxin-related factor to an animal or human body. The invention also provides for a method of treatment comprising administering an antibody which specifically binds a relaxin-related factor to an animal or human body. The relaxin-related factor may be RRF-1 or RRF-2.

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The invention further provides for a relaxin-related factor ligandbody which

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comprises a relaxin-related factor fused to an immunoglobulin constant region. The relaxin-related factor may be RRF-1 or RRF-2. In one embodiment of the relaxin-related factor ligandbody, the immunoglobulin constant region is the Fc portion of human IgG1. The invention further provides for a method of treatment, comprising administering a relaxin-related factor ligandbody to a human or animal body.

As used herein, the term "relaxin-related factor" includes the RRF-1 and RRF-2 molecules described herein, as well as functionally equivalent molecules in which amino acid residues are substituted for residues within the sequence resulting in a silent change. For example, one or more amino acid residues within the sequence can be substituted by another amino acid of a similar polarity which acts as a functional equivalent, resulting in a silent alteration. Substitutes for an amino acid within the sequence may be selected from other members of the class to which the amino acid belongs. For example, the nonpolar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan and methionine. The polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine. The positively charged (basic) amino acids include arginine, lysine and histidine. The negatively charged (acidic) amino acids include aspartic acid and glutamic acid. Also included within the scope of the term "relaxin-related factor" are fragments or derivatives thereof which exhibit the same or similar biological activity and derivatives which are differentially modified during or after translation, *e.g.*, by glycosylation, proteolytic cleavage, linkage to an antibody molecule or other cellular ligand, etc.

One skilled in the art will also recognize that the present invention provides DNA and RNA sequences that hybridize to a relaxin-related factor encoding sequence, under conditions of moderate stringency, as defined in, for example, Sambrook, et al. *Molecular Cloning: A Laboratory Manual*, 2 ed. Vol. 1, pp. 101-104, Cold Spring Harbor Laboratory Press (1989). Thus, a nucleic acid molecule contemplated by the invention includes one having a nucleotide sequence

deduced from an amino acid sequence of a relaxin-related factor as described herein, as well as a molecule having a sequence of nucleotides that hybridizes to such a nucleic acid under conditions of moderate stringency, and also a nucleotide sequence which is degenerate of the above sequences as a result of the 5 genetic code, but which nonetheless encodes a relaxin-related factor.

A nucleic acid encoding a relaxin-related factor as described herein will facilitate the genetic engineering of cells to produce the relaxin-related factor by, e.g., transfection, transduction, electroporation, or microinjection of a suitable host 10 cell using a nucleotide sequence encoding the relaxin-related factor in a suitable expression vector, thus forming a host cell vector system for the production of a polypeptide having the biological activity of the relaxin-related factor. A suitable host cell may be selected from the group consisting of bacterial cells (such as E. coli), yeast cells, fungal cells, insect cells and animal cells. Suitable animal cells 15 include, but are not limited to, Vero cells, HeLa cells, Cos cells, CV1 cells and various primary mammalian cells.

Any of the methods known to one skilled in the art for the insertion of DNA 20 fragments into a vector may be used to construct expression vectors encoding the relaxin-related factor using appropriate transcriptional/translational control signals and the protein coding sequences. These methods may include in vitro recombinant DNA and synthetic techniques and in vivo recombinations (genetic recombination). Expression of a nucleotide sequence encoding relaxin-related 25 factor or peptide fragments thereof may be regulated by a second nucleotide sequence so that the protein or peptide is expressed in a host transformed with the recombinant DNA molecule. For example, expression of the relaxin-related factor described herein may be controlled by any promoter/enhancer element known in the art. Promoters which may be used to control expression of the relaxin-related factor include, but are not limited to the long terminal repeat as 30 described in Squinto et al., (Cell 65:1-20 (1991)); the SV40 early promoter region (Bernoist and Chambon, Nature 290:304-310), the CMV promoter, the M-MuLV 5' terminal repeat, the promoter contained in the 3' long terminal repeat of Rous

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sarcoma virus (Yamamoto, et al., *Cell* 22:787-797 (1980)), the herpes thymidine kinase promoter (Wagner et al., *Proc. Natl. Acad. Sci. U.S.A.* 78:144-1445 (1981)), the adenovirus promoter, the regulatory sequences of the metallothionein gene (Brinster et al., *Nature* 296:39-42 (1982)); prokaryotic expression vectors such as the 5 β -lactamase promoter (Villa-Kamaroff, et al., *Proc. Natl. Acad. Sci. U.S.A.* 75:3727-3731 (1978)), or the tac promoter (DeBoer, et al., *Proc. Natl. Acad. Sci. U.S.A.* 80:21-25 (1983)), see also "Useful proteins from recombinant bacteria" in *Scientific American*, 242:74-94 (1980); promoter elements from yeast or other fungi such as 10 the Gal 4 promoter, the ADH (alcohol dehydrogenase) promoter, PGK (phosphoglycerol kinase) promoter, alkaline phosphatase promoter, and the following animal transcriptional control regions, which exhibit tissue specificity and have been utilized in transgenic animals: elastase I gene control region which is active in pancreatic acinar cells (Swift et al., *Cell* 38:639-646 (1984); Ornitz et al., *Cold Spring Harbor Symp. Quant. Biol.* 50:399-409 (1986); MacDonald, 15 *Hepatology* 7:425-515 (1987); insulin gene control region which is active in pancreatic beta cells (Hanahan, *Nature* 315:115-122 (1985)), immunoglobulin gene control region which is active in lymphoid cells (Grosschedl et al., 1984, *Cell* 38:647-658; Adames et al., 1985, *Nature* 318:533-538; Alexander et al., 1987, *Mol. Cell. Biol.* 7:1436-1444), mouse mammary tumor virus control region which is 20 active in testicular, breast, lymphoid and mast cells (Leder et al., 1986, *Cell* 45:485-495), albumin gene control region which is active in liver (Pinkert et al., 1987, *Genes and Devel.* 1:268-276), alpha-fetoprotein gene control region which is active in liver (Krumlauf et al., 1985, *Mol. Cell. Biol.* 5:1639-1648; Hammer et al., 1987, *Science* 235:53-58); alpha 1-antitrypsin gene control region which is active in the 25 liver (Kelsey et al., 1987, *Genes and Devel.* 1:161-171), beta-globin gene control region which is active in myeloid cells (Mogram et al., 1985, *Nature* 315:338-340; Koliass et al., 1986, *Cell* 46:89-94); myelin basic protein gene control region which is active in oligodendrocyte cells in the brain (Readhead et al., 1987, *Cell* 48:703-712); myosin light chain-2 gene control region which is active in skeletal muscle 30 (Shani, 1985, *Nature* 314:283-286), and gonadotropic releasing hormone gene control region which is active in the hypothalamus (Mason et al., 1986, *Science* 234:1372-1378). The invention further provides for the production of antisense

compounds which are capable of specifically hybridizing with a sequence of RNA encoding the relaxin-related factor to modulate its expression. (Ecker, U.S. Patent No. 5,166,195, issued November 24, 1992).

5 Thus, according to the invention, expression vectors capable of being replicated in a bacterial or eukaryotic host comprising a nucleic acid encoding a relaxin-related factor are used to transfect a host and thereby direct expression of such nucleic acid to produce the relaxin-related factor, which may then be recovered in a biologically active form. As used herein, a biologically active form includes a 10 form capable of binding to a receptor for the relaxin-related factor and causing a biological response such as a differentiated function or influencing the phenotype of the cell expressing the receptor.

Expression vectors containing the gene inserts can be identified by four general 15 approaches: (a) DNA-DNA hybridization, (b) presence or absence of "marker" gene functions, (c) expression of inserted sequences and (d) PCR detection. In the first approach, the presence of a foreign gene inserted in an expression vector can be detected by DNA-DNA hybridization using probes comprising sequences that are homologous to an inserted relaxin-related factor encoding gene. In the 20 second approach, the recombinant vector/host system can be identified and selected based upon the presence or absence of certain "marker" gene functions (e.g., thymidine kinase activity, resistance to antibiotics, transformation phenotype, occlusion body formation in baculovirus, etc.) caused by the insertion of foreign genes in the vector. For example, if a nucleic acid encoding relaxin-related factor is inserted within the marker gene sequence of the vector, 25 recombinants containing the insert can be identified by the absence of the marker gene function. In the third approach, recombinant expression vectors can be identified by assaying the foreign gene product expressed by the recombinant. Such assays can be based, for example, on the physical or functional properties of 30 a relaxin-related factor gene product, for example, by binding of the relaxin-related factor to its receptor or portion thereof which may be tagged with, for example, a detectable antibody or portion thereof or by binding to antibodies

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produced against the relaxin-related factor or a portion thereof. Cells of the present invention may transiently, or preferably constitutively and permanently, express relaxin-related factor as described herein. In the fourth approach, DNA nucleotide primers can be prepared corresponding to a relaxin-related factor specific DNA sequence. These primers could then be used to PCR a relaxin-related factor gene fragment. (PCR Protocols: A Guide To Methods and Applications, Edited by Michael A. Innis et al., Academic Press (1990)).

A recombinant relaxin-related factor may be purified by any technique which allows for the subsequent formation of a stable, biologically active protein. For example, and not by way of limitation, a relaxin-related factor may be recovered from cells either as soluble proteins or as inclusion bodies, from which they may be extracted quantitatively by 8M guanidinium hydrochloride and dialysis. In order to further purify the relaxin-related factor, conventional ion exchange chromatography, hydrophobic interaction chromatography, reverse phase chromatography or gel filtration may be used.

In additional embodiments of the invention, a recombinant relaxin-related factor encoding gene may be used to inactivate or "knock out" the endogenous gene by homologous recombination, and thereby create a relaxin-related factor deficient cell, tissue, or animal. For example, and not by way of limitation, the recombinant relaxin-related factor encoding gene may be engineered to contain an insertional mutation, for example the neo gene, which would inactivate the native relaxin-related factor encoding gene. Such a construct, under the control of a suitable promoter, may be introduced into a cell, such as an embryonic stem cell, by a technique such as transfection, transduction, or injection. Cells containing the construct may then be selected by G418 resistance. Cells which lack an intact relaxin-related factor encoding gene may then be identified, e.g. by Southern blotting, PCR detection, Northern blotting or assay of expression. Cells lacking an intact relaxin-related factor encoding gene may then be fused to early embryo cells to generate transgenic animals deficient in such relaxin-related factor. Such an animal may be used to define specific in vivo processes,

normally dependent upon the relaxin-related factor.

The present invention also provides for antibodies to a relaxin-related factor described herein which are useful for detection of the factor in, for example, diagnostic applications. For the preparation of monoclonal antibodies directed toward a relaxin-related factor, any technique which provides for the production of antibody molecules by continuous cell lines in culture may be used. For example, the hybridoma technique originally developed by Kohler and Milstein (1975, *Nature* 256:495-497), as well as the trioma technique, the human B-cell hybridoma technique (Kozbor et al., 1983, *Immunology Today* 4:72), and the EBV-hybridoma technique to produce human monoclonal antibodies (Cole et al., 1985, in "Monoclonal Antibodies and Cancer Therapy," Alan R. Liss, Inc. pp. 77-96) and the like are within the scope of the present invention.

15 Monoclonal antibodies for diagnostic or therapeutic use may be human monoclonal antibodies or chimeric human-mouse (or other species) monoclonal antibodies. Human monoclonal antibodies may be made by any of numerous techniques known in the art (e.g., Teng et al., 1983, *Proc. Natl. Acad. Sci. U.S.A.* 80:7308-7312; Kozbor et al., 1983, *Immunology Today* 4:72-79; Olsson et al., 1982, *Meth. Enzymol.* 92:3-16). Chimeric antibody molecules may be prepared containing a mouse antigen-binding domain with human constant regions (Morrison et al., 1984, *Proc. Natl. Acad. Sci. U.S.A.* 81:6851, Takeda et al., 1985, *Nature* 314:452).

25 Various procedures known in the art may be used for the production of polyclonal antibodies to epitopes of a relaxin-related factor described herein. For the production of antibody, various host animals can be immunized by injection with a relaxin-related factor, or a peptide fragment or derivative thereof, including but not limited to rabbits, mice and rats. Various adjuvants may be used to increase the immunological response, depending on the host species and including, but not limited to, Freund's adjuvant (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as

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lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanins, dinitrophenol, and potentially useful human adjuvants such as BCG (Bacille Calmette-Guerin) and Corynebacterium parvum.

5 A molecular clone of an antibody to a selected relaxin-related factor epitope can be prepared by known techniques. Recombinant DNA methodology (see e.g., Maniatis et al., 1982, Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York) may be used to construct nucleotide sequences which encode a monoclonal antibody molecule, or antigen 10 binding region thereof.

The present invention provides for antibody molecules as well as fragments of such antibody molecules. Antibody fragments which contain the idiotype of the molecule can be generated by known techniques. For example, such fragments 15 include but are not limited to: the $F(ab')_2$ fragment which can be produced by pepsin digestion of the antibody molecule; the Fab' fragments which can be generated by reducing the disulfide bridges of the $F(ab')_2$ fragment; and the Fab fragments which can be generated by treating the antibody molecule with papain and a reducing agent. Antibody molecules may be purified by known techniques, 20 e.g., immunoabsorption or immunoaffinity chromatography, chromatographic methods such as HPLC (high performance liquid chromatography), or a combination thereof.

Having isolated a nucleic acid molecule encoding a relaxin-related factor, it 25 should pose no great difficulty to create an expression construct that would yield a secreted protein consisting of the entire coding sequence of the relaxin-related factor fused to the human immunoglobulin gamma-1 constant region (IgG1 Fc). These fusion proteins are called "relaxin-related factor ligandbodies." The relaxin-related factor may be RRF-1 or RRF-2.

30 The Fc portion may be prepared as follows. A DNA fragment encoding the Fc portion of human IgG1 that spans from the hinge region to the carboxy-terminus

15

of the protein may be amplified from human placental cDNA by PCR with oligonucleotides corresponding to the published sequence of human IgG1; the resulting DNA fragment may be cloned into a plasmid vector. Appropriate DNA restriction fragments from a plasmid encoding the full-length relaxin-related factor and from the human IgG1 Fc plasmid may be ligated on either side of a short PCR-derived fragment that is designed so as to fuse, in-frame, the chosen relaxin-related factor with human IgG1 Fc protein-coding sequences.

10 The present invention therefore also provides for a relaxin-related factor ligandbody which specifically binds the chosen relaxin-related factor's receptor. In one embodiment, the present invention provides for a relaxin-related factor ligandbody which comprises a relaxin-related factor ligand fused to an immunoglobulin constant region. In a further embodiment, the ligandbody comprises a relaxin-related factor and the Fc portion of human IgG1. In a further 15 embodiment, the ligandbody comprises RRF-1 and the Fc portion of human IgG1. In yet another embodiment, the ligandbody comprises RRF-2 and the Fc portion of human IgG1. A relaxin-related factor ligandbody as described above may be used in a method of treatment of the human or animal body, or in a method of diagnosis.

20 By way of nonlimiting example, relaxin-related factor ligandbodies may be useful for the delivery of toxins to a receptor bearing cell. Alternatively, other molecules, such as growth factors, cytokines or nutrients, may be delivered to a relaxin-related factor receptor bearing cell via relaxin-related factor ligandbodies. 25 A relaxin-related factor ligandbody could also be used as a diagnostic reagent for a relaxin-related factor receptor, to detect a receptor in vivo or in vitro. A relaxin-related factor ligandbody may be useful as a diagnostic reagent for detecting a relaxin-related factor receptor by, for example, tissue staining or whole body imaging.

30 The present invention also provides for pharmaceutical compositions comprising a relaxin-related factor ligand or ligandbody described herein, peptide

16

fragments thereof, or derivatives in a pharmacologically acceptable vehicle. The relaxin-related factor protein, or peptide fragments or derivatives thereof, may be administered systemically or locally. Any appropriate mode of administration known in the art may be used including, but not limited to, intravenous, 5 intrathecal, intraarterial, intranasal, oral, subcutaneous, intraperitoneal, or by local injection or surgical implant. Sustained release formulations are also provided for.

This invention will be better understood from the Examples which follow. 10 However, one skilled in the art will readily appreciate that the specific methods and results discussed are not limiting but are merely illustrative of the invention.

15 EXAMPLE 1 - CLONING OF A MOLECULE RELATED TO RELAXIN AND THE INSULIN FAMILY OF LIGANDS

A search of the database of expressed sequence tags using the sequence of relaxin (a member of the insulin family, which also includes insulin, IGF1, IGF2, and 20 "Leydig insulin-like protein") as the test sequence revealed the existence of a sequence, found in human testis, related to relaxin at the amino acid level. The hallmarks of the insulin family of proteins consist mainly of a particular arrangement of cysteine residues, two near the amino terminus and four towards the carboxy terminus, which form a characteristic structure of disulfide bonds. 25 The sequence found on the database contained the first two cysteines, along with a few other residues in their immediate vicinity that are also characteristic of the insulin family. This homology was rather weak, and the presence of a stop codon in the middle of the database sequence, with no evidence for the characteristic C-terminal cysteines, raised further doubts about whether this sequence represented 30 an authentic new insulin family member.

Oligonucleotides were synthesized corresponding to the database sequence and

were assembled by PCR to constitute a 400-base probe. A human testis cDNA library was constructed and then screened with this synthetic probe. Several clones resulting from this screen were sequenced, and it was found that at least one of the clones contained an open reading frame whose predicted protein sequence displayed all the expected features of a new insulin family member, particularly with regard to the cluster of four cysteines at the C-terminus of the molecule. The nucleotide and deduced amino acid (single letter code) sequences of the molecule are shown in Figure 1. Overall, the molecule is more closely related to relaxin than to any of the other insulin family members. The molecule was therefore called relaxin-related factor-1 (RRF-1). Figure 2 shows a comparative alignment of the relaxin-related factor-1 amino acid sequence with the sequences of other members of the insulin family.

15 Northern blot analysis was performed by running samples of adult human poly A RNA on a 1% agarose-formaldehyde gel, transferring to a nylon membrane and probing with a 400 base pair probe derived from the nucleotide sequence of the relaxin-related factor. As shown in Figure 3, the message for RRF-1 was highly expressed in human testis, but was not detected in any of the other tissue samples that were assayed, including brain, cerebellum, thymus, heart, liver, 20 small intestine, skeletal muscle, bone marrow, prostate, ovary, uterus, and placenta. Because production of RRF-1 appears to be specific to testis, it may be involved in the maturation of sperm and therefore may have a role in treatment of fertility disorders.

25

EXAMPLE 2 - EXPRESSION OF HUMAN RELAXIN-RELATED FACTOR-1 cDNA

30 Expression vectors were constructed containing the human relaxin-related factor-1 cDNA. One version had a triple-myc tag inserted at its C-terminus to make the protein detectable. COS-7 cells were transiently transfected with either the expression vector or a control vector by the DEAE-dextran transfection protocol.

18

Briefly, COS-7 cells were plated at a density of 1.0×10^6 cells per 100 mm plate 24 hours prior to transfection. For transfection, the cells were cultured in serum-free DMEM containing 400 μ g/ml of DEAE-dextran, 1 μ M chloroquine, and 2 mM glutamine, and 1 μ g of the appropriate DNA for 3-4 hours at 37°C in an atmosphere of 5% CO₂. The transfection media was aspirated and replaced with phosphate-buffered saline with 10% DMSO for 2-3 min. Following this DMSO "shock", the COS-7 cells were placed into DMEM with 1% each of penicillin and streptomycin, and 2 mM glutamine. COS media containing secreted ligand was harvested after three days. Expression of the myc-tagged version was verified by 10 Western blot analysis of the supernatants, using antibodies against the myc tag as a probe.

EXAMPLE 3 - CLONING OF A SECOND MOLECULE RELATED
TO THE INSULIN FAMILY OF LIGANDS

15 A second search of the database of expressed sequence tags using the sequence of relaxin as the test sequence revealed the existence of numerous sequence entries, all found in human placental cDNA and representing essentially the same sequence, which was related to relaxin at the amino acid level. As stated 20 previously, the hallmarks of the insulin family of proteins consist mainly of a particular arrangement of cysteine residues, two near the amino terminus and four towards the carboxy terminus, which form a characteristic structure of disulfide bonds. The human placental sequences found on the database contained the last four cysteines, along with a few other residues in their 25 immediate vicinity that are also characteristic of the insulin family. This homology was rather weak, and there was variation among the different entries on the database, with some having frameshift mutations with respect to others, making it somewhat unclear whether the homology represented an authentic new insulin family member. In addition, it was noticed that many of these 30 sequences had a stretch that was identical to the cDNA sequence for a known ribosomal protein, L22, and these sequence entries were identified on the database as L22, further obscuring the homology with relaxin and supporting the

possibility that the relaxin homology was artifactual. Further examination revealed that the L22 protein was transcribed in the opposite direction from the relaxin-homologous sequence, so it was possible that there were two overlapping genes transcribed from opposite strands, and splicing mechanisms may have included portions of one cDNA in the other.

5 Oligonucleotides were synthesized corresponding to parts of the database sequence and were used to screen a human placental cDNA library. One clone resulting from this screen was sequenced, and it was found that it contained an 10 open reading frame whose predicted protein sequence displayed many of the expected features of a new insulin family member: there was a signal sequence at the amino terminus, allowing for the secretion of the protein, followed by a sequence with two cysteines separated by 11 amino acid residues, as is the case with all other insulin family members. In addition, there was a tetrabasic 15 sequence just preceding the C-terminal four-cysteine cluster, which is present in relaxin and is probably involved in proteolytic processing of the protein (other members of the family have dibasic residues at this position). Although these sequence signatures identify this new molecule as an insulin family member, the sequence in the vicinity of the amino-terminal cysteine cluster is rather more 20 distant from all the others, and therefore the new molecule may be thought of as identifying a new branch of the insulin family. Overall, the new molecule is more closely related to relaxin than to any of the other insulin family members. The molecule was therefore called relaxin-related factor-2 (RRF-2). Figure 2 25 shows a comparative alignment of the relaxin-related factor-2 amino acid sequence with the sequences of RRF-1 and other members of the insulin family.

Northern blot analysis was performed as previously described in Example 1 and the results, shown in Figure 5, revealed that the message for relaxin-related factor-2 (RRF-2) was highly expressed in human placenta, but was not detected in 30 any of the other samples that were assayed. Because production of RRF-2 appears to be specific to placenta, it may be useful for modulating the reproductive physiology of mammals during pregnancy and parturition.

5 EXAMPLE 4 - EXPRESSION OF HUMAN RELAXIN-RELATED FACTOR-2
cDNA

10 Expression vectors were constructed containing the human relaxin-related factor-2 cDNA. One version had a FLAG tag inserted at its N-terminus to make the protein detectable. COS-7 cells were transiently transfected with either the expression vector or a control vector by the DEAE-dextran transfection protocol. Expression of the FLAG-tagged version was verified by staining of the cells using antibodies against the FLAG tag as a probe.

15 The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and accompanying Figures. Such modifications are intended to fall within the scope of the appended claims.

WHAT IS CLAIMED IS:

1. An isolated nucleic acid molecule encoding a relaxin-related factor.
2. An isolated nucleic acid molecule according to claim 1, having a sequence selected from the group consisting of:
 - (a) the nucleotide sequence comprising the coding region of the relaxin-related factor as set forth in Figure 1;
 - (b) a nucleotide sequence that hybridizes under moderately stringent conditions to the nucleotide sequence of (a) and which encodes a molecule having the biological activity of the relaxin-related factor; or
 - (c) a nucleotide sequence which, but for the degeneracy of the genetic code would hybridize to a nucleotide sequence of (a) or (b), and which encodes a molecule having the biological activity of the relaxin-related factor.
3. A vector which comprises a nucleic acid molecule of claim 1 or 2.
4. A vector according to claim 3, wherein the nucleic acid molecule is operatively linked to an expression control sequence capable of directing its expression in a host cell.
5. A plasmid according to claim 3 or 4.
6. Isolated relaxin-related factor substantially free of other proteins.
7. Isolated relaxin-related factor-1 substantially free of other proteins, encoded by a nucleic acid molecule according to claim 2.
8. A host-vector system for the production of a relaxin-related factor which comprises a vector of claim 3 or 4, in a host cell.

9. A host-vector system according to claim 8, wherein the host cell is a bacterial, yeast, insect or mammalian cell.
10. A method of producing a relaxin-related factor which comprises growing cells of a host-vector system of claim 8 or 9, under conditions permitting production of the relaxin-related factor, and recovering the relaxin-related factor so produced.
11. An antibody which specifically binds the relaxin-related factor of claim 6 or 7.
12. An antibody according to claim 11, which is a monoclonal antibody.
13. A pharmaceutical composition comprising a relaxin-related factor according to claim 6 or 7, and a pharmaceutically acceptable carrier.
14. A pharmaceutical composition comprising an antibody according to claim 11 or 12, and a pharmaceutically acceptable carrier.
15. A relaxin-related factor according to claim 6 or 7, an antibody according to claim 11 or 12, or a composition according to claim 13 or 14, for use in a method of treatment of the human or animal body, or in a method of diagnosis.
16. A polypeptide produced by the method of claim 10.
17. A ligandbody which comprises relaxin-related factor-1 fused to an immunoglobulin constant region.
18. The ligandbody of claim 17, wherein the immunoglobulin constant region is the Fc portion of human IgG1.

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19. A ligandbody according to claim 17 or 18, for use in a method of treatment of the human or animal body, or in a method of diagnosis.
20. An isolated nucleic acid molecule according to claim 1, having a sequence selected from the group consisting of:
 - (a) the nucleotide sequence comprising the coding region of the relaxin-related factor as set forth in Figure 4;
 - (b) a nucleotide sequence that hybridizes under moderately stringent conditions to the nucleotide sequence of (a) and which encodes a molecule having the biological activity of the relaxin-related factor; or
 - (c) a nucleotide sequence which, but for the degeneracy of the genetic code would hybridize to a nucleotide sequence of (a) or (b), and which encodes a molecule having the biological activity of the relaxin-related factor.
21. A vector which comprises a nucleic acid molecule of claim 20.
22. A vector according to claim 21, wherein the nucleic acid molecule is operatively linked to an expression control sequence capable of directing its expression in a host cell.
23. A plasmid according to claim 21 or 22.
24. Isolated relaxin-related factor-2 substantially free of other proteins, encoded by a nucleic acid molecule according to claim 20.
25. A host-vector system for the production of a relaxin-related factor which comprises a vector of claim 21 or 22, in a host cell.
26. A host-vector system according to claim 25, wherein the host cell is a

bacterial, yeast, insect or mammalian cell.

27. A method of producing a relaxin-related factor which comprises growing cells of a host-vector system of claim 25 or 26, under conditions permitting production of the relaxin-related factor, and recovering the relaxin-related factor so produced.
28. An antibody which specifically binds the relaxin-related factor of claim 24.
29. An antibody according to claim 28, which is a monoclonal antibody.
30. A pharmaceutical composition comprising a relaxin-related factor according to claim 24, and a pharmaceutically acceptable carrier.
31. A pharmaceutical composition comprising an antibody according to claim 28 or 29, and a pharmaceutically acceptable carrier.
32. A relaxin-related factor according to claim 24, an antibody according to claim 28 or 29, or a composition according to claim 30 or 31, for use in a method of treatment of the human or animal body, or in a method of diagnosis.
33. A polypeptide produced by the method of claim 27.
34. A ligandbody which comprises relaxin-related factor-2 fused to an immunoglobulin constant region.
35. The ligandbody of claim 34, wherein the immunoglobulin constant region is the Fc portion of human IgG1.
36. A ligandbody according to claim 34 or 35, for use in a method of treatment of the human or animal body, or in a method of diagnosis.

Fig. 1.

10	20	30	40	50	60
*	*	*	*	*	*
CCATTGTGCT	GGAAAGCTCG	GCCTGGCGCA	GGGCCAGACA	GGGAGCAGGG	TCCGGGCCGAC
GGTAACACGA	CCTTTCGAGC	CGGACCCGGT	CGCGGTCTGT	CCCTCGTCCC	AGGCCGGCTG
70	80	90	100	110	*
*	*	*	*	*	*
CGCCATTGCA	CAACGGGAG	GAATAGCCCTG	GGGTCAACAGG	GATG CCG CGG	CTC CTC
GCGTAACGT	GTTGGCCTC	CTGATCGAC	CCCAGTGTCC	TAC GCC GAG	GAG
R	L	S	L	M	P
120	130	140	150	160	*
*	*	*	*	*	*
CGC TTC	CTG CTG	TGG CTT	GGA CTC	CTG CTG	GTT CGG TTT
GCG AAC	AGG GAC	ACC GAC	CCT GAG	GAC GAC	AAA GCC
R	L	S	L	L	V
170	180	190	200	210	*
*	*	*	*	*	*
GAA CTG	AGC GAC	ATC AGC	AGT GCC	AGG AAG	CTG TGC
CTT GAC	TCG CTG	CTG TAG	TCA CGG	TCC TTC	GGC AGG
E	L	S	D	I	A
220	230	240	250	260	*
*	*	*	*	*	*
GTG AAA	GAA ATA	GAA AAA	CTC TGC	GGC CAT	GCC AAC
CAC TTT	CTT TAT	CTT TTT	GAG ACG	CCG GTA	TGG ACC
V	K	E	I	E	K

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Fig. 1 (Cont 1/3).

270	*	*	280	*	*	290	*	*	300	*	*				
CGT	TTC	GAG	GAG	ACC	CCT	TTC	TCA	CGG	TTG	ATT	GCA	CAG	GCC	TCG	
GCA	AAG	CTC	CTC	CTC	CTT	TGG	GGA	AAG	AGT	GCC	AAC	TAA	CGT	GTC	
R	F	E	E	E	T	P	F	S	R	L	I	A	Q	AGC	
310	*	*	320	*	*	330	*	*	340	*	*	350	*	*	
GAG	AAG	GTC	GAA	GCC	TAC	AGC	CCA	TAC	CAG	TTC	GAA	AGC	CCG	CAA	ACC
CTC	TTC	CAG	CTT	CGG	ATG	TGG	GGT	ATG	GTC	AAG	CTT	TCG	GGC	GTT	TGG
E	K	V	E	A	Y	S	P	Y	Q	F	E	S	P	Q	T>
360	*	*	370	*	*	380	*	*	390	*	*	400	*	*	
GCT	TCC	CCG	CCC	GGG	AGA	GGC	ACA	AAC	CCA	GTG	TCT	ACT	TCT	TCT	TGG
CGA	AGG	GGC	CCC	GGG	CCT	TCT	CCG	TGT	TTC	GGT	CAC	AGA	TGA	AGA	ACC
A	S	P	A	R	G	R	G	T	N	P	V	S	T	S	W>
410	*	*	420	*	*	430	*	*	440	*	*	450	*	*	
GAA	GAA	GCA	GTA	AAC	AGT	TGG	GAA	ATG	CAG	TCA	CTA	CCT	GAG	TAT	AAG
CTT	CTT	CGT	CAT	TTG	TCA	ACC	CTT	TAC	GTC	AGT	GAT	GGA	CTC	ATA	TTC
E	E	A	V	N	S	W	E	M	Q	S	L	P	E	Y	K>
460	*	*	470	*	*	480	*	*	490	*	*	500	*	*	
GAT	AAA	AAG	GGA	TAT	TCA	CCC	CTT	GGT	AAG	ACA	AGA	GAA	TTT	TCT	TCA
CTA	TTT	TTC	CCT	ATA	AGT	GGG	GAA	CCA	TTC	TGT	TCT	CTT	AAA	AGA	AGT
D	K	K	G	Y	S	P	L	G	K	T	R	E	F	S	S>

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Fig. 1 (Cont 2/3).

510	*	*	520	*	*	530	*	*	540	*	*				
TCA	CAT	AAT	ATC	AAT	GTA	TAT	ATT	CAT	GAG	AAT	GCA	AAA	TTT	CAG	AAG
AGT	GTA	TTA	TAG	TTA	CAT	ATA	TAA	GTA	CTC	TTA	CGT	TTT	AAA	GTC	TTC
S	H	N	I	N	V	Y	I	H	E	N	A	K	F	Q	K>
550	*	*	560	*	*	570	*	*	580	*	*	590	*	*	*
AAA	CGT	AGA	AAC	AAA	ATT	AAA	ACC	TTA	AGC	AAT	TG	TTT	TGG	GGG	CAT
TTT	GCA	TCT	TTG	TTT	TTA	TTT	TGG	ATT	TCG	TTA	AAC	AAA	ACC	CCC	GTA
K	R	N	K	I	K	T	L	S	N	L	F	W	G	H>	
600	*	*	610	*	*	620	*	*	630	*	*	640	*	*	*
CAT	CCC	CAA	AGA	AAA	CGC	AGA	GGA	TAT	TCA	GAA	AAG	TGT	TGT	CTT	ACA
GTA	GGG	GTT	TCT	TTT	GGC	TCT	CCT	ATA	AGT	CTT	TTC	ACA	GAA	TGT	
H	P	R	K	R	R	G	Y	S	E	K	C	C	L	T>	
650	*	*	660	*	*	670	*	*	680	*	*	690	*	*	*
GGA	TGT	ACA	AAA	GAA	GAA	CTT	AGC	ATT	GCA	TGT	CTT	CCA	TAT	ATT	GAT
CCT	ACA	TGT	TTT	CCT	CTT	GAA	TCG	TAA	CGT	ACA	GAA	GGT	ATA	TAA	CTA
G	C	T	K	E	E	L	S	I	A	C	L	P	Y	I	D>

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Fig. 1 (Cont 3/3).

700	*	*	*	*	*	*	*	*	*	*	*	*				
*	TTT	AAA	AGG	CTA	AAG	GAA	AAA	AGA	TCA	TCA	CTT	GTA	ACT	AAG	ATA	TAC
F	K	R	L	K	E	K	R	S	S	L	V	T	K	I	Y>	
710	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
*	TTT	TTT	TCC	GAT	TTC	CTT	TTT	TCT	AGT	AGT	GAA	CAT	TGA	TTC	TAT	ATG
*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
720	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
*	TAACC	CAT	CTT	AGA	ATT	TTT	TTT	CTA	AAAC	CTTAAT	AAAAG	CTTTC	CAGCT	TTCCA	GCACAA	TGG
*	GGT	AGA	TTT	TTT	TTT	TTT	TTT	TTT	TTT	TTT						
730	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
*	ATT	GGT	AGA	TTT	TTT	TTT	TTT	TTT	TTT	TTT	TTT	TTT	TTT	TTT	TTT	TTT
740	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
*	TTT	TTT	TTT	TTT	TTT	TTT	TTT	TTT	TTT	TTT	TTT	TTT	TTT	TTT	TTT	TTT
*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	
750	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
*	TA	AC	CA	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT
*	CC	AT	CT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT
760	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
*	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT
770	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
*	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT
780	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
*	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT
790	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
*	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT

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Fig.2.

RRF-1 M (SIGNAL SEQUENCE) -----RKLICGRYLVKETEKLKG (123aa) RKR ----GYSER CCLTGCTKEELSIAACLPHYDDFKRLKEKRSSSLVTKLY
 RRP-2 M (SIGNAL SEQUENCE) -----ELRG . PRFGKHLSSY.P (66aa) RKKR SGRHREDFP . EVI . DDGTSVKL.T

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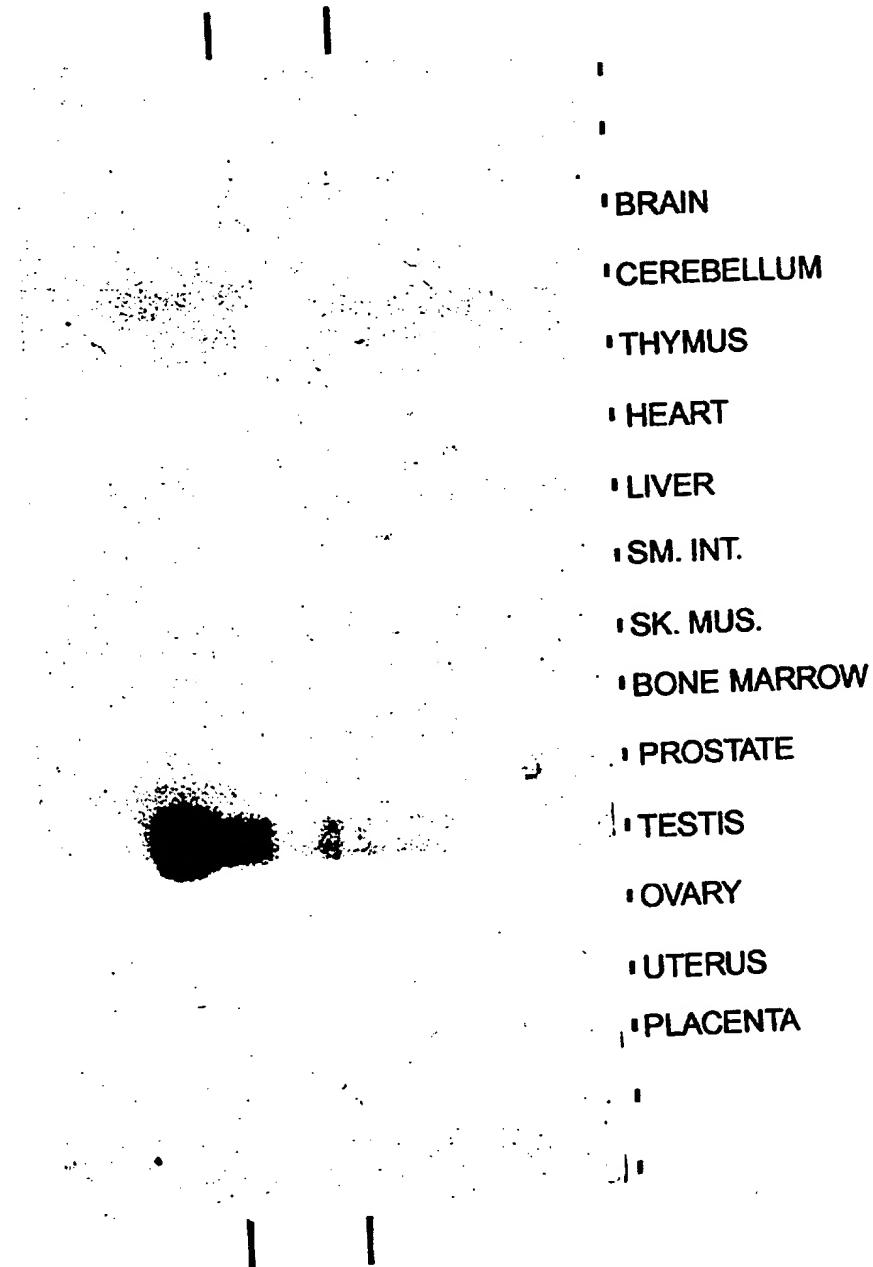
RELAXINH1 (SIGNAL SEQUENCE) KWKDDV1 E . RAQIAI
 RELAXINH2 (SIGNAL SEQUENCE) -WMEEV1 E . RAQIAI
 LEYDIG (SIGNAL SEQUENCE) PTPEMRE HHF . RALVRV
 INSULIN (SIGNAL SEQUENCE) ---FVNQH SH . EALYLV
 IGF1 (SIGNAL SEQUENCE) --TAGPET AE . DALQFV
 IGF2 (SIGNAL SEQUENCE) --YRPSET GE . DTLQFV

SHEET (RUL) (SIGNAL SEQUENCE) (110aa) KERR P-YVALP . - RS . AKY .
 (SIGNAL SEQUENCE) (109aa) RKKR QLYSALAN RS . ARF .
 (SIGNAL SEQUENCE) (54aa) RHHR AAATNPARY S QD . LTL
 (SIGNAL SEQUENCE) (43aa) KR-- ---GIVHQ TSI . SLYQ . ENY .
 (SIGNAL SEQUENCE) (16aa) RR-- APQTGTVD FRS . DLRR . EMY . (86aa)
 (SIGNAL SEQUENCE) (14aa) RR-- SR-- QTVB FRS . DLAL . STY . (96aa)

(26)

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Fig 3.



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Fig.4.

10 20 30 40 50 60 70 80

* * * * * * * *

CCATTGGCT GGAAGGACA CACAGGACA GTCTGGTAGG CTACAGGAGC AAGTCTCTAA AGAAAGGCTG AGAACACCCA

GGTAACACGA CCTTTCCTGT GTGTCGTGT CAGACCATCC GATGTCGTCG TTCAAGAGATT TCTTTCGGAC TCTTGTGGCT

90 100 110 120 130 140

* * * * * *

GAACAGGAGA GTTCAGGTCC AGG ATG GCC AGC CTG TTC CGG TCC TAT CTG CCA GCA ATC TGG CTG CTG

CTTGTCTCT CAAGTCCAGG TCC TAC CGG TCG GAC AAG GCC AGG ATA GAC GGT CGT TAG ACC GAC GAC

M A S L F R S Y L P A I W L L>

150 160 170 180 190 200 210

* * * * * * *

CTG AGC CAA CTC CTT AGA GAA AGC CTA GCA GAG CTG AGG GGA TGT GGT CCC CGA TTT GGA AAA

GAC TCC GTT GAG GAA TCT CTT TCG GAT CGT CGT CTC GAC TCC CCT ACA CCA GGG GCT AAA CCT TTT

L S Q L L R E S L A A E L R G C G P R F G K>

220 230 240 250 260 270 280

* * * * * * *

CAC TTG CTG TCA TAT TGC CCC ATG CCT GAG AAG ACA TTC ACC ACC ACC CCA GGA GGG TGG CTG CTG

GTG AAC GAC AGT ATA ACG GGG TAC GGA CTC TTC TGT AAG TGG TGG CCT CCC ACC GAC GAC

H L L S Y C P M P E K T F T T P G G W L L>

290 300 310 320 330 340

* * * * * *

GAA TCT GGA CGT CCC AAA GAA ATG GTG TCA ACC TCC AAC AAC AAA GAT GGA CAA GCC TTA GGT ACG

CTT AGA CCT GCA GGG TTT CTT CTT TAC CAC AGT TGG AGG TTG TTG CCT CCT GGT CGG AAT CCA TGA

E S G R P K E M V S T S N N K D G Q A L G T>

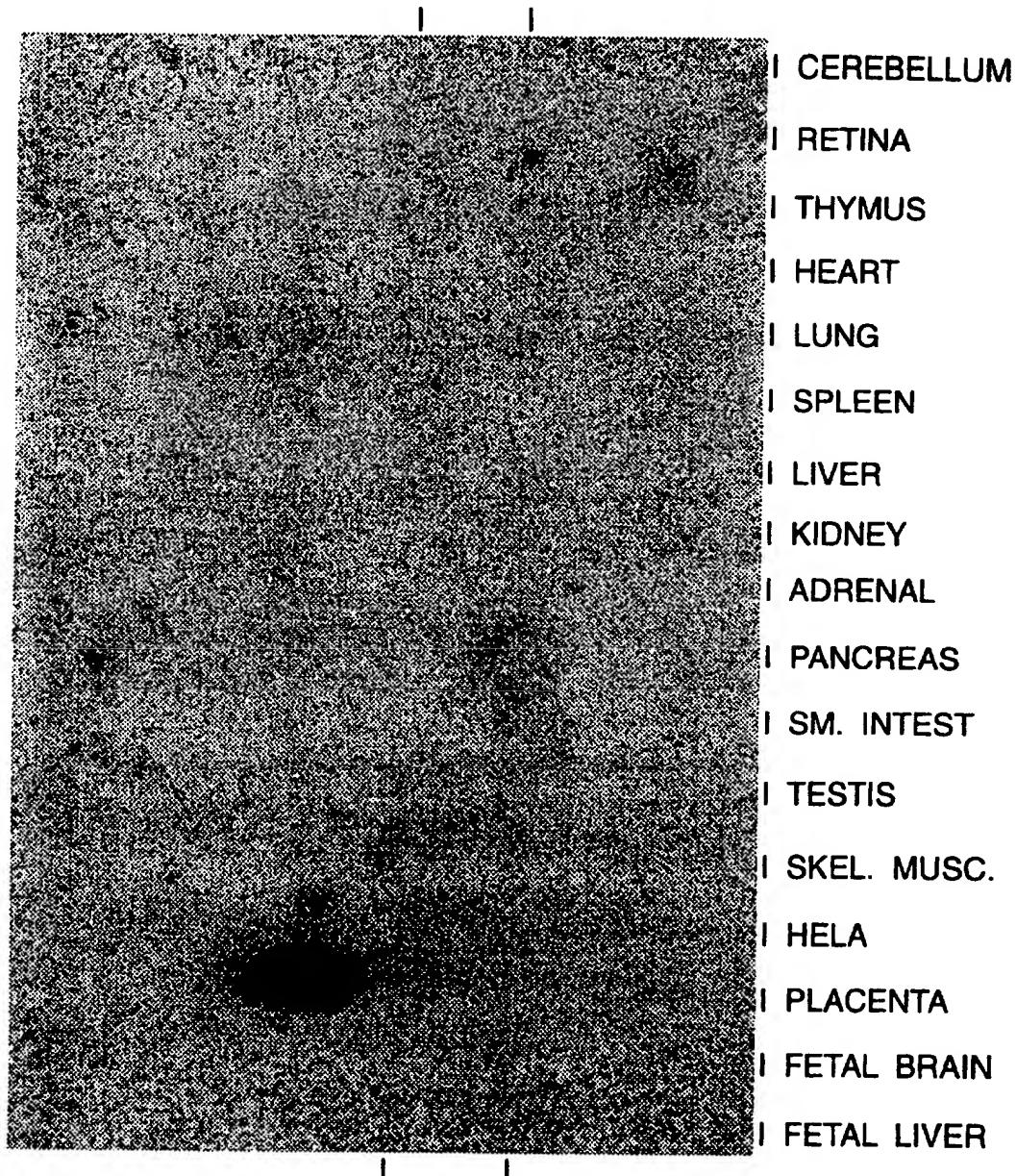
Fig.4 (Cont).

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350	*	360	*	370	*	380	*	390	*	400	*	410	*									
ACA	TCA	GAA	TTC	ATT	CCT	AAT	TTC	TCA	CCA	GAG	CTG	TCT	GAA	GGG	CAG	CCA	TCA					
TGT	AGT	CCT	AAG	TAA	GGA	TTA	AAC	AGT	GGT	CTC	GAC	TTC	TTT	GGT	GAC	AGA	CTT	CCC	GTC	GGT	AGT	
T	S	E	F	I	P	N	L	S	P	E	L	K	K	P	L	S	E	G	Q	P	S>	
420	*	*	*	*	*	*	*	*	*	*	440	*	*	450	*	*	460	*	*	470	*	
TTG	AAG	AAA	ATA	ATA	CTT	TCC	CGC	AA	AAG	AGA	AGT	GGA	CGT	CAC	AGA	TTT	GAT	CCA	TTC	TGT	TGT	
AAC	TTC	TTT	TAT	TAT	GAA	AGG	GCG	TTT	TTC	TCT	TCA	CCT	GCA	GTC	TCT	AAA	CTA	GGT	AAG	ACA	ACA	
L	K	K	I	I	L	S	R	K	K	R	S	G	R	H	R	F	D	P	F	C	C>	
480	490	*	*	*	*	500	*	*	*	*	510	*	*	520	*	*	530	*	*	540	*	550
GAA	GTA	ATT	TGT	GAC	GAT	GGG	ACT	TCA	GTT	AAA	TTA	TGT	ACA	TAGTAGAGTA	ATCATGGACT	GGACATCTCA						
CTT	CAT	TAA	ACA	CTG	CTA	CCT	TGA	AGT	CAA	TTT	AAT	ACA	TGT	ATCATCTCAT	TAGTACCTGA	CCTGTAGAGT						
E	V	I	C	D	G	T	S	V	K	L	C	T>										
560	570	*	*	*	*	580	*	*	*	*	590	*	*	600	*	*	610	*	*	620	*	630
TCCATTCTCA	TATGTATTCT	CAATGACAAA	TTCACTGATG	CCCAATTAAA	TGATTGCTGT	TTTATTAGAAC	ATGAAAAAAA															
AGGTAAAGT	ATACATAAGA	GTTACTGTT	AAGTGACTAC	GGGTTAATT	ACTAACGACA	ATAATCTTG	TACTTTTG															
640	*	*	*	*																		
AAACTTTCCA	GCACAAATGG																					
TTTGAAGGT	CGTGTACCC																					

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Fig.5.





INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(72) Inventor; and (75) Inventor/Applicant (for US only): DAVIS, Samuel [US/US]; Apartment #B2, 332 W. 88th Street, New York, NY 10024 (US).			
(74) Agents: COBERT, Robert, J.; Regeneron Pharmaceuticals, Inc., 777 Old Saw Mill River Road, Tarrytown, NY 10591 (US) et al.			
(54) Title: MOLECULAR CLONING AND CHARACTERIZATION OF MOLECULES RELATED TO RELAXIN AND THE INSULIN FAMILY OF LIGANDS			
(57) Abstract <p>This invention concerns the molecular cloning and characterization of nucleic acids encoding molecules, referred to as relaxin-related factors, that are related to the hormone relaxin and to the insulin family of ligands. More specifically, this invention provides an isolated nucleic acid molecule encoding relaxin-related factor-1. The invention also provides an isolated nucleic acid molecule encoding relaxin-related factor-2. The present invention also provides a nucleotide sequence that encodes a relaxin-related factor, as well as cells which are genetically engineered to produce the relaxin-related factor. The invention further provides for an isolated relaxin-related factor substantially free of other proteins, as well as for an antibody which specifically binds the relaxin-related factor. The invention also provides for pharmaceutical compositions comprising a relaxin-related factor, or an antibody which specifically binds the relaxin-related factor, and a pharmaceutically acceptable carrier, as well as for methods of treatment of a human or animal body. The invention further provides for a relaxin-related factor ligandbody which comprises a relaxin-related factor fused to an immunoglobulin constant region. The invention further provides for a method of treatment comprising administering a relaxin-related factor ligandbody to a human or animal body.</p>			

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 96/17342

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/16 C07K14/64 C12N5/10 C12N1/21 C12N1/19
C07K16/26 A61K38/22 A61K39/395 C07K19/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GENOMICS 29 (2). 1995. 465-470. ISSN: 0888-7543, 20 September 1995, XP000644716 CHASSIN D ET AL: "Cloning of a new member of the insulin gene superfamily (INSL4) expressed in human placenta." see the whole document ---	1,2
A	NATURE, vol. 301, 1983, pages 628-631, XP002025421 P. HUDSON ET AL: "Structure of a genomic clone encoding biologically active human relaxin" -----	

 Further documents are listed in the continuation of box C. Patent family members are listed in annex.

* Special categories of cited documents :

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- 'L' document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- 'O' document referring to an oral disclosure, use, exhibition or other means
- 'P' document published prior to the international filing date but later than the priority date claimed

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- 'X' document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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Date of the actual completion of the international search 19 February 1997	Date of mailing of the international search report 16. 05. 97
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl. Fax: (+31-70) 340-3016	Authorized officer VAN DER SCHAAL C.A.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 96/17342

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:

2. Claims Nos.: because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

5 inventions * see continuation-sheet PCT/ISA/210 *

1. As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

2,7,17-19 and, partially, 1,3-6,8-16

Remark on Protest

The additional search fees were accompanied by the applicant's protest.

No protest accompanied the payment of additional search fees.